

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	: Confirmation No. 4204
Serial No. 10/560,936	: Group Art Unit: 1781
Kenzo Takahashi	: Examiner: GWARTNEY,
Filed: May 16, 2006	ELIZABETH A

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patent and Trademarks

Sir:

I, Hideki Maki, declare that:

I am not an inventor of the above-identified US patent Application, but an inventor of U.S. patent application serial No.11/933052(US2011/0104358A1);

I received the master's degree in 1996, received from Agriculture from the Nagoya University;

I have been an employee of Suntory Holdings Limited, Japan, since 1996 up to the present and have been engaged in the research of tea beverages for more than 15 years;

I am an inventor of the following patent applications;

1. Nagao, Koji et al., US 2008/138489 A1
2. Nagao, Koji et al., US 2010/272857 A1
3. Terazawa, Noriko et al., US 2011/014347 A1
4. Terazawa, Noriko et al., WO 2011/055789 A1

5. Hiroki, Furuta et al., JP 2009-219447A
6. Shinichi, Kobayashi et al., JP 2010-063432A
7. Terazawa, Noriko et al., JP 2010-088396A
8. Hideki, Maki et al., JP 2010-207116A
9. Shinichi, Kobayashi et al., JP 2011-010640A
10. Shinichi, Kobayashi et al., JP 2011-010641A

The experiment set out below was conducted under my supervision and direction.

## 1. Method

### (1) Preparation of ground tea dispersion

Tencha (a kind of green tea) leafs were ground under the following conditions to prepare ground tea leafs:

- (1) tea leafs ground with a stone mortar and having an average particle size of 15  $\mu\text{m}$
- (2) tea leafs ground with a stone mortar and having an average particle size of 11  $\mu\text{m}$
- (3) tea leafs ground with a cutter mill and having an average particle size of 45  $\mu\text{m}$ .
- (4) tea leafs ground with a cutter mill and having an average particle size of 210  $\mu\text{m}$ .

200 mL of warm water (35°C) was added to 0.4g of these ground tea leafs, respectively, and the mixtures were left for 5 minutes to obtain powdered tea suspensions. These suspensions were centrifuged to remove solid contents having a large particle size, then cooled down to 20°C to obtain ground

tea dispersions.

## (2) Quantitative analysis of glyceroglycolipids

Thereafter, insoluble solid contents were removed at room temperature by use of a membrane filter (with a pore size of 0.45  $\mu$ m, JUJI FIELD INC., unsterilized water system 13A). Then, pressure filtration was performed by use of an ultrafiltration membrane (Millipore, Biomax PBMK ultrafiltration disk, polyether sulfone, 300,000 NMWL), and a component on the membrane was recovered to obtain a taste (kokumi) enhanced component having a molecular weight of at least 300,000. This was dissolved in water, acidified with HCl, and subjected to liquid-liquid distribution with ethyl acetate. Among the products of the liquid-liquid distribution, an ethyl acetate layer was adsorbed onto an ODS solid-phase extraction cartridge (Waters, Sep-Pak Plus C18), and eluted in 100% ethanol.

The eluted liquid was subjected to reverse chromatography, and quantitative analysis of glyceroglycolipids was performed. Analysis conditions are as follows:

Reverse column: TSK-GEL (TOSOH, inner diameter 4.6 mm. x length 150 mm)

Amount of introduced sample: 10  $\mu$ l

Flow rate: 1.0 ml/min

RI detector: RIA-10A of SHIMADZU

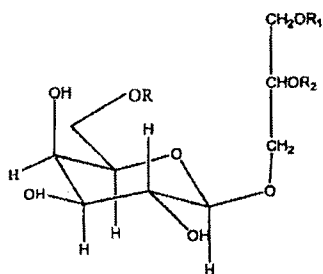
Eluant: 95% methanol

Temperature: 40°C

MGDG and DGDG of Lipid Products were used as preparations

in the quantitative analysis. Among the preparations, MGDG is divided roughly into two peaks; since a peak exhibited in the ultrafine ground tea dispersion was a succeeding peak, concentrations were prorated on the basis of proportions of the peak areas of the preparations to determine analysis concentrations. A total amount of MGDG and DGDG was used to indicate a concentration of glyceroglycolipids.

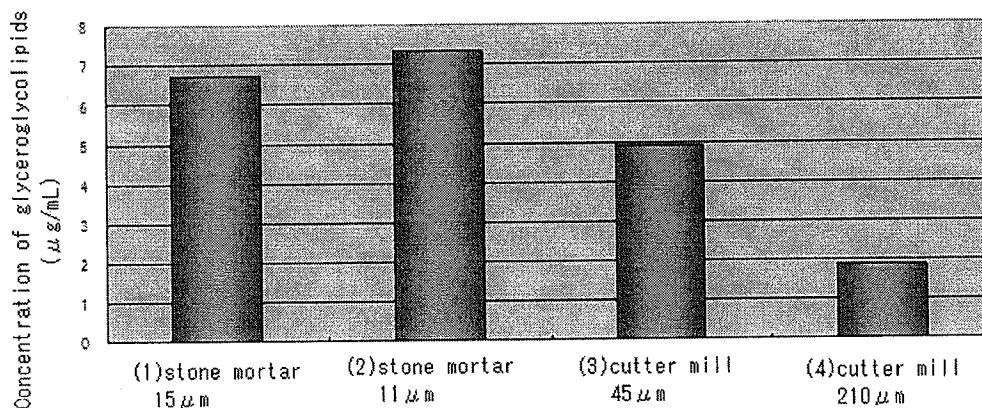
The structures of Glyceroglycolipids analyzed by NMR are shown below.



wherein  $R_1$  and  $R_2$  are fatty acids, and  $R$  is hydrogen atom or galactose.

## 2. Result

The amounts of glyceroglycolipids are shown below.



As is clear from the above figure, the amount of glyceroglycolipids in the ground tea dispersion prepared using a stone mortar is larger than that using a cutter mill.

It is declared by the undersigned that all statements made herein of undersigned's own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: October 5, 2011

Hideki Maki

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